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# Evaluation of a Commercial Measles Virus Immunoglobulin M Enzyme Immunoassay

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**Paired serum samples from 93 patients suspected of having measles were assayed for measles virus-specific immunoglobulin M (IgM) antibodies by an enzyme immunoassay (EIA), and the results were compared with results from a complement fixation assay and an EIA for measles virus IgG. By using significant serologic rises as the standard for comparison, the IgM EIA assay had a sensitivity of 85.7%, a specificity of 81.3%, a positive predictive value of 95.7%, and a negative predictive value of 54.2%. This assay can be expected to perform well in outbreak situations.**

The incidence of measles in the United States has increased in the last several years. Despite heightened vaccination efforts and recommendations concerning a second vaccine dose, the current measles epidemic continues (2). The impact of the resurgence of measles on diagnostic laboratories has been great, as evidenced by the 10-fold increase in requests for measles virus antibody tests in this laboratory between 1988 and 1989. The increased demand for testing continued into 1990. Accordingly, there were 9 measles cases serologically diagnosed in this laboratory in 1988, 129 diagnosed in 1989, and 99 diagnosed in 1990. The standard laboratory procedure for serologic diagnosis of viral disease usually involves testing of acute- and convalescent-phase sera. Testing of a single serum sample for the presence of measles virus immunoglobulin M (IgM) would greatly speed up timely diagnosis, but until recently such assays have not been commercially available in this country. This report is a laboratory evaluation of a commercial measles virus enzyme immunoassay (EIA) IgM kit which was used to test paired serum samples from 93 patients suspected of having measles virus infection.

Paired serum samples from individuals throughout Connecticut who were suspected of having acute measles virus infection were collected between December 1989 and July 1990. Sufficient quantities of paired sera to run a measles virus EIA for IgM, a measles virus EIA for IgG, and a complement fixation (CF) assay for measles virus antibody were gathered from 93 patients. There were 48 males and 43 females in this group. Gender was not given for two patients. Information on the date of onset of rash and measles vaccination status was obtained when available from clinical case reports submitted to the Connecticut Immunizations Program or to the state laboratory. This information was used to calculate the time interval between onset of rash and collection of serologic specimens. Characteristics of confirmed measles cases with positive IgM tests were compared with those with false-negative IgM tests to determine predictors of false negativity. Odds ratios were calculated, and the statistical significance of them was determined by Fisher's exact test.

Indirect EIA kits commercially available from Whittaker Bioproducts Inc. (Walkersville, Md.) were used to detect anti-measles virus IgG (Measelisa II) and anti-measles virus IgM (Measlestat M). The manufacturer's directions were followed exactly. A significant serologic rise was indicated when the convalescent-phase EIA titer divided by the acute-phase EIA titer exceeded 1.47. CF studies were done by using a microtiter adaptation method (1). A fourfold or greater difference between the titers of acute- and convalescent-phase sera was considered significant. For the purposes of this evaluation, a patient was considered to be positive for recent measles infection if a significant rise in value was demonstrated by either CF assay or IgG EIA. This was the reference against which the results of the IgM EIA were compared. A patient was considered positive by the IgM EIA if a significant IgM value was found in either the acute- or the convalescent-phase serum sample. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated as described by Griner et al. (4).

A serologic profile of the 93 pairs of serum samples from individuals suspected of having acute measles infection is summarized in Table 1. In total, 77 patients had a rise in titer for CF or IgG (serologically confirmed measles cases). Of the 77 serologically confirmed measles cases, 66 were positive for measles IgM. Sixty-four of these 66 IgM-positive patients (97%) had IgM detectable in the convalescent-phase serum sample of their serum pairs. That is, only two cases were positive for IgM in the acute-phase serum sample and negative in the convalescent-phase serum sample. The number of true-positive IgM cases was 66, the number of true negatives was 13, the number of false positives was 3, and the number of false negatives was 11. Therefore, the sensitivity of the IgM assay was 85.7%, the specificity was 81.3%, the positive predictive value was 95.7%, and the negative predictive value was 54.2%.

The timing of specimen collection for IgM testing relative to rash onset was found to be a critical determinant of test sensitivity. Figure 1 shows the distribution of IgM results of each serum sample ($n$ = 128) from IgM-positive, serologically confirmed measles cases. For four serum samples, the exact dates of collection after onset of suspected measles were unknown; these samples were therefore excluded from Fig. 1. By day 1 and 2 after onset of illness, more than half of the IgM-positive measles patients had one serum sample positive for IgM. By day 6 (or more) after onset, virtually every serum sample from this group was IgM positive (63 of 64). However, the positivity rate for specimens collected

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TABLE 1. Summary of serologic findings for paired serum samples from 93 individuals suspected of having acute measles infection

| Serologic results | No. of pairs | IgM test status |
|---|---|---|
| CF titer rise[a], IgG titer rise[b], IgM positive[c] | 62[d] | True positive |
| CF stable, IgG titer rise, IgM positive | 4 | True positive |
| CF titer rise, IgG titer rise, IgM negative | 8 | False negative |
| CF stable, IgG titer rise, IgM negative | 3 | False negative |
| CF stable, IgG stable, IgM positive | 3 | False positive |
| CF stable, IgG stable, IgM negative | 13 | True negative |

[a] Fourfold or greater titer rise.
[b] Convalescent-phase value/acute value ≥ 1.47.
[c] Value ≥ 0.17.
[d] Includes one pair with high values of 128 and 256 by CF, values of 0.81 and 0.84 by IgG EIA, and values of 0.21 and 0.36 by IgM EIA.

within 5 days of rash onset was 56.3% (36 of 64) compared to a rate of 98.4% (63 of 64) for those collected later ($P$ = 0.001).

It might be expected that previously vaccinated individuals would be more likely than unvaccinated individuals to respond to measles infection with a rise in IgG rather than IgM, thereby giving a false-negative IgM response. However, when IgM-negative patients were compared with IgM-positive patients for previous vaccination status, no evidence of an effect of previous vaccination could be found to explain the false IgM negativity. Only 1 of 7 patients false negative for IgM in whom vaccination status was known had been vaccinated, compared to 18 of 51 IgM-positive cases (odds ratio = 0.31; $P$ = 0.41). These results agree with those of a previous study which found no significant effect of vaccination on the presence or absence of measles virus IgM after serologically confirmed measles infection (3). We were unable to determine whether the 11 patients false negative for IgM had their acute- and convalescent-phase sera drawn before the IgM test could be expected to be positive, because 8 of them did not have precise dates of rash onset recorded.

The false-negative rate of 14.3% was one limitation of the test. Although we were unable to identify factors for false IgM negativity, we were able to rule out previous vaccination as a possible explanation for it. It is noteworthy that the 14.3% false-negative rate is similar to the 16% rate found in another study (3). Other assays have been used with varying success for the detection of measles virus-specific IgM. These include the standard hemagglutination inhibition test, in-house EIAs, and indirect fluorescent antibody assays (3, 5, 6).

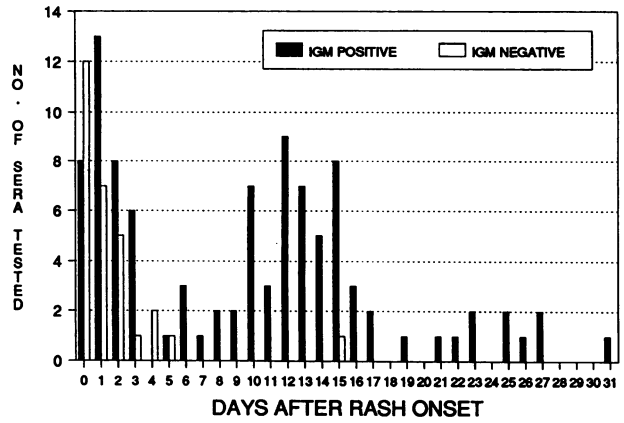


FIG. 1. Appearance of measles IgM in each serum sample from IgM-positive patients.

The three false-positive IgM test results were examined for the timing and nature of their IgG responses. For one patient, the acute- and convalescent-phase sera were drawn only 1 day apart, 4 and 5 days after rash onset, respectively. Both the CF tests and the IgG EIAs were nonreactive, while the IgM test results were values of 0.23 and 0.35. For a second patient, when the two serum samples were drawn could not be determined. However, the CF test showed a twofold rise, and the IgG EIA resulted in values of 0.14 and 0.19, respectively. In a third patient, the two specimens were taken 1 and 16 days after the onset of rash. Although the IgM test readings were 0.20 and 0.37 on the two tests, the CF test and IgG EIA were both nonreactive.

Although as many as three false-positive IgM tests were identified by our criteria, only one of them was clearly false positive. The interpretation of the other two was complicated by the fact that the IgG tests were either performed too early to ensure their sensitivity as being a "gold standard" or showed slight but consistent increases that did not quite meet the criteria for significance. In addition, in the latter case, the interval between collection dates of serum samples was unknown. Thus, while our official false-positive rate is reported as 18.8% (3 of 16 true negatives), it could be as low as 7.1% (1 of 14). In a separate study, we tested 100 serum samples from normal, healthy individuals which had been collected and frozen at −70°C in 1988, before the current measles epidemic. Three of these 100 were positive (data not shown). We also tested 100 serum samples that had been collected in 1990 during the epidemic from normal healthy individuals, and 10 of these were positive (data not shown). We assume that the true false-positive rate is somewhere between 3 and 10%, although we did not independently test each false-positive serum sample to verify the absence or presence of measles virus-specific IgM. A positive IgM value after testing the sera from healthy individuals may result from subclinical infection or vaccination.

The major limitation of this study is the lack of a perfect gold standard for definition of measles cases. Neither the CF tests nor the IgG EIAs are necessarily 100% sensitive, especially when used in the practical outbreak setting. The timing of acute- and convalescent-phase serum collection is often less than optimal both in terms of onset of rash and relative to each other. A second limitation is that assessment of the use of the test was limited to diagnosis of cases of rash illness during a measles outbreak. In this setting, in which at least 77 of 93 paired serum samples tested positive for measles virus, the positive predictive value of the test was very high, 95.7%. In a setting in which fewer tested specimens were truly positive for measles virus, the positive predictive value would be lower.

We believe that measles virus IgM laboratory tests with the performance characteristics of this one, when used appropriately, can be an important early indicator of measles disease in any setting and can potentially result in saving considerable laboratory time in the outbreak setting. However, we recommend several qualifications to full dependence on it. First, during outbreaks, its positive predictive value is sufficient for a positive result to be considered truly positive without the need for additional confirmation. In settings of sporadic measles case activity, however, positive

IgM tests should be verified with acute- and convalescent-phase IgG tests. Second, its sensitivity is such that negative IgM tests in clinically or epidemiologically suspect measles cases should be backed up with testing of acute- and convalescent-phase sera for significant IgG titer rises.

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